## REMARKS

Claims 73-80 have been canceled. Claims 73-80 were drawn to methods for detecting a disease or a disease susceptibility trait associated with a germline mutation in one subject gene, and wherein the calculation of the ratio of the amount of wild-type protein expressed by the subject gene is with reference to the amount of wild-type protein expressed by a reference gene **not** known to be associated with a disease or a disease susceptibility trait. In a previous Office Action dated July 17, 2001, the Examiner required restriction to one of two inventions under 35 USC 121:

> I. Claims 1-6, 9-23, and 45-53, drawn to methods for detecting a disease or a disease susceptibility trait associated with a germline mutation in one subject gene, classified in class 435, subclass 7.1.
>
> II. Claims 24-28, 31-44 and 54, drawn to methods for detecting a disease or a disease susceptibility trait associated with a germline mutation in one of two or more subject genes, classified in class 435, subclass 7.1.

[7/17/01 Office Action, page 3.] In the Preliminary Amendment and Response to Second Restriction Requirement and Request for Provisional Election of Species dated August 15, 2001, Applicant elected the invention of Group II, and cancelled the claims of Group I.

Due to an inadvertent oversight, in the Amendment and Response to Office Action dated March 3, 2004, Applicant mistakenly entered new claims 73-80, directed to methods for detecting a disease or a disease susceptibility trait associated with a germline mutation in only one subject gene. Claims 73-80 then belong to the invention of Group I of the 7/17/01 restriction requirement. Therefore, Applicant has cancelled claims 73-80 without prejudice, reserving the right to prosecute the claims of Group I in a subsequent divisional application.

Claim 24 has been amended to point out with more particularity and clarity the subject matter regarded by the Applicant as his invention. The preamble of independent Claim 24, from which Claims 25-72 depend, has been amended to clarify

that the candidate disease or disease susceptibility trait is one that has been associated with a 50% reduction of wild-type protein expressed by a subject gene in affected individuals relative to the level of the same wild-type protein expressed in unaffected individuals. General support for that amendment can be found in the instant specification at least as follows:

> The assays of this invention are based on the assumption that gene expression directly relates to gene dosage, that is, the presence of two wild-type alleles will result in the expression of twice the amount of full-length wild-type protein than would occur if only one wild-type allele were present.

[Instant application, page 2, lines 24-27.]

> The immunoassays of this invention may be adapted to measure full-length (wild-type) proteins associated with **many other hereditary and genetic disorders (cancer and non-cancer)** that are due to mutations that cause protein truncation (germline and acquired) or cause the absence of allelic protein expression.

[Instant application, page 7, lines 6-8; emphasis added.]

Particular support for the amendments to Claim 24 can be found in Example 1, as follows:

> Anti-APC antibodies were used in this study to immunoprecipate full-length APC from the lymphocytes of FAP patients, who are known to have one mutant and one remaining wild-type APC allele in their germline. **This study supports the theory underlying the immunoassays of this invention in that the lymphoblastoid cells from the FAP patients were shown to have about 50% less (50.1% ± 5.1%) immunoprecipitatable full-length APC protein in comparison to controls lacking germline APC mutations**. The results correlate with the heterozygous APC genotypic status in FAP cells.

[Instant application, page 45, lines 22-29; emphasis added.]

> Described in this example is the first study (to the inventor's present knowledge) which shows that APC mutations lead to a decrease in the expression of full-length APC protein. This is likely to have biological relevance because the amount of

> full-length APC protein in colonocytes is probably a critical factor in mechanisms underlying colon tumorigenesis. For example, it was previously hypothesized . . . that an APC mutation involving one APC allele may render cells more susceptible to the deleterious effects on cellular growth that are induced by other acquired genetic changes or by carcinogenic substances. The present results indicate that a reduced level of full-length APC protein actually occurs as a consequence of an APC mutation involving one APC allele.
>
> **Quantitatively, the results show that the level of anti-APC antibody immunoprecipitable protein in the FAP lymphoblastoid cell lines was about 50% of normal controls. That level of immunoreactivity is consonant with the fact that FAP patients carry only one wild-type APC allele along with one mutant allele, and with the presumption that gene product expression is proportional to gene dosage.**
>
> **This example using FAP cells as a model system indicates that the immunoassays of this invention are useful to detect germline mutations that reduce target full-length protein levels.** This example supports such assays as a simple, reliable, low cost way to diagnose individuals carrying a deleterious, mutant allele. Immunoassays for levels of full-length APC levels should be useful as a practical diagnostic test for detecting individuals affected with FAP.

[Instant application, page 49, line 27 to page 50, line 11; emphasis added.]

Applicant respectfully submits that no new matter has been entered by the above amendments.

**Withdrawal of 103(a) Rejections in Sections 4-8 of Office Action**

Applicant respectfully acknowledges, appreciates and agrees with the Examiner's withdrawal of the 35 USC 103(a) rejections, based each in view of Nozawa, on Vogelstein (Section 4), Markowitz (Section 5), Liskay (Section 6), Tavtigian (Section &) and Albertsen (Section 8).

**35 USC 112, 1st Paragraph Rejection (Section 9 of Office Action)**

"Known to be associated with"

Claims 24-28, 32-35, 37-44, 55-57, and 59-80 stand rejected under 35 USC 112, first paragraph on the basis that "[t]he claim(s) contains subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." [Office Action, page 3, section 9.] Applicant respectfully traverses that rejection, pointing out that independent Claim 24 has been amended to point out with more particularity and clarity the subject matter regarded by the Applicant as his invention. Applicant further respectfully points out that the instant rejection no longer applies to claims 73-80 which have been cancelled.

The Examiner states at page 4 of the Office Action, that

> the disclosure fails to describe the genus of disease or disease-susceptibility traits that are "known" to be associated with a germline mutation that causes an about 50% decrease in the level of wild-type protein normally expressed by one of two or more subject genes. . . .
>
> It appears that one would have to perform the claimed assay in order to "know" that a disease or disease-susceptibility trait is associated with a germline mutation that causes an about 50% decrease in the level of wild-type protein.

Applicant respectfully traverses, pointing out that first, Claim 24 has been amended to clarify the genus of disease or disease-susceptibility traits that are the subject of the present invention, as conveyed in the instant specification; and second, that the subject genus of disease or disease-susceptibility traits, while described in the specification, is not defined by the claimed assay.

The preamble of Claim 24 now reads:

> A method of detecting a disease or a disease susceptibility trait in an organism, wherein said disease or said disease susceptibility trait has been associated with a germline mutation that causes an about 50% decrease in the level of wild-type protein expressed by one of two or more subject genes in affected individuals relative to the level of said wild-

> type protein normally expressed by the same subject gene in unaffected individuals, wherein said germline mutation is selected from the group consisting of truncation-causing mutations, mutations that cause allelic loss, and mutations that cause the expression of proteins with non-wild-type epitopes, and wherein each of said subject genes has been associated with such a disease or such a disease susceptibility trait, . . .

[Claim 24; emphasis added.] The preamble of Claim 24 has been amended, to clarify that the disease or disease susceptibility trait that is the subject of the present invention is a disease or disease susceptibility trait that has been associated with a germline mutation which results in an about 50% reduction in one gene product in affected individuals, relative to the level of the same gene product in unaffected individuals. The specification clearly indicates that that is the genus of disease or disease susceptibility that is the subject of the instant invention, as indicated above in the support for the amendments to Claim 24.

That such haploinsufficient[2] diseases exist, is already established. The methods that have been used to detect such hereditary diseases are conventional; that is, DNA sequence analysis and/or in vitro translation-type assays, to determine the responsible germline mutation in affected individuals, which mutation has been frequently found to result in allelic loss. As Example 1 from the specification demonstrates, protein assays can be performed that further support the theory that gene product levels are reduced by about half (by the loss of one allele) in affected individuals, relative to control individuals[3]; but such protein assays are not examples of the claimed assay.

---

2. **Haploinsufficiency:** "a locus shows haploinsufficiency if producing a normal phenotype requires more gene product than the amount produced by a single copy." [Strachan and Read, Human Molecular Genetics 2, John Wiley & Sons, Inc., New York, 1999; www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=hmg.glossary.]

3. In fact, although additional factors can alter the protein levels in the cell (e.g., feedback mechanisms, mRNA turnover, etc.), in most cases gene product levels correspond to gene dosage: "[I]n general, the amount of transcript produced by a gene is directly proportional to the number of copies of that gene in a cell. That is, for a given gene, the rate of transcription is directly related to the number of DNA templates." [Griffiths et al., An Introduction to Genetic Analysis, Seventh

The protein assay **of the preamble** of Claim 24, which is based on the theory of haploinsufficiency and compares the protein levels found in affected individuals directly with the protein levels found in normal individuals, is also based on a premise that the **absolute level of one wild-type protein above a threshold** is the critical factor for determining deleterious gene dosage effects on phenotype in an individual. Applicant respectfully distinguishes such a protein assay from the **claimed assay**; although also based on the theory of haploinsufficiency, the **claimed assay** is based instead on the premise that the **ratio of two wild-type proteins within one individual** is the critical factor for deleterious gene dosage effects on phenotype, and that that ratio is a more promising index of haploinsufficiency than absolute levels of one wild-type protein, in a comparison of individuals in affected and unaffected populations.

The amendment to the preamble of Claim 24 clarifies the distinction between the assays which define the subject genus of disease or disease susceptibility traits, and the assays of the instant invention:

> A method of detecting a disease or a disease susceptibility trait in an organism, wherein said disease or said disease susceptibility trait has been associated with a germline mutation that causes an about 50% decrease in the level of wild-type protein expressed by any of two or more subject genes in affected individuals relative to the level of said wild-type protein normally expressed by the same subject genes in unaffected individuals, . . . wherein each of said subject genes is known to be associated with such a disease or such a disease susceptibility trait, comprising. . . .

[Emphasis added.] However, as the specification indicates at page 50, the protein assay of the preamble of Claim 24 is not the best protein-based assay to detect gene dosage effects:

> Due to the variability in sample loading and to other confounding factors, the approach used determines the individual expression level of each of the two proteins, and then the ratio of one to the other is calculated. Then it is determined whether the numerical value of the ratio falls clearly in the normal range, or clearly in the range predicted

---

Ed., W. H. Freeman, New York, (2000). www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=iga, Chapter 18, "Mechanisms of Gene Imbalance".]

if there were 50% loss of expression of one of the two proteins.

[Instant application, page 50, line 31 to page 51, line 3]

Beyond the consideration of variability of sample loading, there are at least two "other confounding factors" which support the premise of the instant invention, that a protein-based assay which uses a ratio of two gene products found in an individual is superior to a protein-based assay based on a direct comparison of the level of one gene product found in affected and control individuals. Those two factors are: 1) potential stoichiometry of interacting proteins produced by the subject genes, and 2) the range of "normal" (i.e., non-deleterious) variation in subject gene product levels.

There is mounting evidence that when looking for a gene dosage effect as a basis of a hereditary disease, the critical parameter may be the correct proportions of gene products, rather than absolute protein levels above a certain threshold. Evidence supporting the theory that several factors in the same pathway may be dosage sensitive, for example, subject to haploinsufficiency because of altered stoichiometry between resulting gene products, is provided in Veitia, R.A., "Exploring the etiology of haploinsufficiency," Bioessays, 24:175-184 (2002) [a copy of which is enclosed, and the citation for which is listed on the accompanying Form PTO A820]: "Even when the phenomenon (of haploinsufficiency) is linked in all cases to reduced absolute gene expression levels, there are several well-documented cases where the explanation is not reduced expression *per se* but altered stoichiometry." [ Id. (Abstract) at p. 175; emphasis added.]

The instant invention is directed to protein-based assays for gene dosage effects that reference measurements of a subject gene product to a second gene product within the same sample, isolated from the same individual, rather than referencing the gene product in question only to the same gene product in unaffected individuals. Therefore, the instant invention is based on a ratio of the levels of two wild-type proteins measured in a sample of one individual in comparison to an average ratio of the same two proteins measured in normal individuals. As stated in the instant application at page 8, lines 18-22, concerning the instantly claimed methods, wherein ratios of the amounts of wild-type proteins of two or more subject genes are calculated:

> The assay could be characterized as a form of differential diagnosis/prognosis, determining in one assay which of several genes is affected by a disease-association mutation.

In sum, the protein assay of the preamble of Claim 24, used to define the genus of diseases to be detected by the protein assay of the invention, is based on a the measurement of one subject protein in affected individuals compared to unaffected individuals. The claimed assay instead uses **a ratio** of two subject proteins in an affected individual compared to an **average ratio** of the same two subject wild-type proteins in unaffected individuals.

Applicant respectfully submits that conventional methods known to those of skill in the art can be used to find germline mutations that are selected from the group consisting of truncation-causing mutations, mutations that cause allelic loss, and mutations that cause the expression of proteins with non-wild-type epitopes, and wherein such a germline mutation is in a subject gene that is associated with a disease or a disease susceptibility trait. Once such a germline mutation in a subject gene is identified, ones of skill in the art would know in view of the instant application, that the claimed assays could be used to screen for such a germline mutation in that subject gene along with such germline mutations in one or more other such subject genes. Such germline mutations could have been identified a long time ago, recently or could yet to be identified. Many examples of such germline mutations in subject genes associate with disease or disease susceptibility are listed in the instant specification. Ones of skill in the art upon the identification of another such germline mutation in a subject gene would then realize the applicability of the claimed assays to screen for that germline mutation in conjunction with screening for one or more other such germline mutations in subject genes.

Applicant respectfully points out that a "specification is directed to those skilled in the art and need not teach or point out in detail that which is well-known in the art." [In re Myers, 161 USPQ 668, 671 (CCPA 1969); see also, G.E. Col. v. Brenner, 159 USPQ 335 (CAFC 1968).] As the Federal Circuit stated in Spectra-Physics, Inc. v.

Coherent, Inc., 3 USPQ2d 1737, 1743 (Fed. Cir. 1987): "A patent need not teach, and preferably omits, what is well known in the art."[4] [Emphasis added.]

The Patent and Trademark Office Board of Appeals and Interference [the "Board"] stated in Ex parte Forman, 230 USPQ 546 at 547 (PTO Bd. App. & Interf. 1986) that the "test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine. . . ." Further, the Board in Ex parte Mark, 12 USPQ 1904 (PTO Bd. Pat. App. & Interf. 1989) reversed an examiner's undue experimentation rejection based on the "limited successful embodiments shown and the established unpredictability associated with . . . site-specific mutageneses . . . to obtain even one biologically active mutein." The Board in reversing pointed out that

> only routine experimentation would be needed for one skilled in the art to practice the claimed invention for any given protein. The fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement. One skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity.

[Id. at 1907; emphasis added.]

As the Federal Circuit stated in In re Certain Limited-Charge Cell Culture Microcarriers, 221 USPQ 1165 at 1174 (U.S. ITC 1983), aff'd sub nom., Massachusetts

---

4. See also, Rengo Co. Ltd. v. Molins Mach. Co., 211 USPQ 303, 319 (3d Cir. 1980) wherein the Third Circuit stated, referring to two CCPA opinions [In re Wiggins, 179 USPQ 421, 424-425 (CCPA 1973) and In re Bode, 193 USPQ 12 (CCPA 1977)]:

> It is axiomatic that no description, however detailed, is "complete" in a rigorous sense. Every description will rely to some extent on the reader's knowledge of the terms, concepts, and depictions it embodies. Thus, an understanding of any description will involve some measure of inference. . . . [S]kill in the art can be relied upon to supplement that which is disclosed as well as to interpret what is written.

Institute of Technology v. AB Fortia, et al., 227 USPQ 428 (Fed. Cir. 1985): "Thus, the fact that experimentation may be complex . . . does not necessarily make it undue, if the art typically engages in such experimentation."

Why should the Applicant disclose his inventive methods to the world, and then be limited to just the particular diseases or disease susceptibilities that he listed in the specification that are presently known to be associated with the subject type of germline mutations? How would such a restriction on the scope of an invention further the goals of the U.S. Constitution "to promote the progress of science and the useful arts by securing for limited times to . . . inventors the exclusive right to their respective . . . discoveries"?

The CCPA in In re Goffe, 191 USPQ 429 at 431 (CCPA 1976) criticized the U.S. Patent and Trademark Office for attempting to limit the appellant to specific claims, lest a competitor seeking to avoid infringement could achieve this goal readily by merely following the disclosure in the patent when it issues. "[A] broad claim can be enabled by the disclosure of a single embodiment." [Precision Metal Fabricators Inc. v. Jetstream Systems Co., 6 USPQ2d 1704, 1709 (N.D. Calif. 1988).]

MPEP § 2164.04 entitled "Burden on the Examiner Under the Enablement Requirement" directs that the initial burden of proof to challenge a presumptively enabling disclosure is upon the Examiner. The patent case law, as well as the MPEP, makes clear that in accordance with case law, statements in a patent specification relied upon for enabling support that correspond in scope to a claimed invention "*must* be taken as in compliance with the enabling requirement of the first paragraph of § 112 *unless* there is reason to doubt the objective truth of" those statements. [In re Marzocchi, 169 USPQ 367, 369 (CCPA 1971); italicized emphasis in the original; underlined emphasis added.] Applicant respectfully submits that there is no reason to doubt the objective truth of statements relied upon for enabling support in the Specification for the claimed invention.

Applicant respectfully points out that at the time of filing an application, an applicant need not have any examples proving a claimed utility. An invention may be constructively reduced to practice by filing an application with no working examples at all or with paper examples. As the Federal Circuit has stated:

> The first paragraph of § 112 requires nothing more than *objective* enablement. *In re Marzocchi*, . . . , 169 USPQ 367, 369 (CCPA 1971). How such a teaching is set forth either by the use of illustrative examples or by broad terminology, is irrelevant.

[In re Vaeck, 20 USPQ2d 1438 at 1445 (Fed. Cir. 1991); emphasis added.]

Applicant respectfully concludes that the instant application reasonably conveys to ones of skill in the art that the Applicant at the time of filing the application had possession of the claimed invention, and that the instant application meets the written description requirement of 35 USC 112, first paragraph. Applicant respectfully requests that the Examiner reconsider and withdraw this rejection in view of the above amendments and remarks.

**35 USC 103(a) Rejections**

Nozawa

Both of the 35 USC 103(a) rejections of Sections 10 and 11 of the Office Action are based on one reference in combination with Nozawa et al., U.S. Patent No. 5,328,826 (issued July 12, 1994; hereinafter cited as "Nozawa"). Applicants respectfully relies upon the remarks and arguments concerning Nozawa in the previous Amendment and Response to Office Action dated March 3, 2004 to address the instant 103(a) rejections.

Applicant again respectfully points out that the instant 103(a) rejections based on Nozawa are not on point in that Nozawa is not analogous art. Nozawa describes the quantification of a cancer antigen isolated from cancer cells, not normal antigen isolated from normal cells. The problems with quantitating antigens for the detection of disease, that Nozawa addresses in the paragraphs to which the Office Action refers, relate to false positives resulting from subjective interpretation of immunohistochemical staining of cells, nonspecific binding of the detecting anti-cancer antigen antibody in normal cells (giving rise to false positives), and the difficulty in collecting samples of a constant volume (and therefore, of a constant cell number) for the cells in question, endometrial cells or tissue from the uterine cavity [Nozawa et al., col. 2, lines 44-51]. None of those problems relates to the present invention.

According to the methods of the present invention, all normal tissues express the antigen in question, that is, the reverse situation from Nozawa. For the preferred type of cells used in the present invention, peripheral blood lymphocytes, a constant volume of cell sample is easily obtainable. The ratio of Nozawa is used to determine the "signal-to-noise" ratio of the specific antigen-antibody staining relative to background staining, in order to eliminate findings of false positives resulting from background staining (due to nonspecificity of antibody, interfering components in the specimen, etc.). Therefore, the cutoff value of Nozawa et al. refers to ratios above a certain "apparent cancer antigen"-to-housekeeping antigen ratio, representing background noise. Claim 5 of Nozawa reads: "wherein a positive result is indicated by a ratio larger than a predetermined value."

The present invention, however, does not use the ratio as a lower limit cutoff, but a defining value of about 50%, indicating that the ratios of Nozawa et al. and the present invention refer to two different phenomena. In a normal cell sample, in the ratio of **y/x** of Nozawa, the signal intensity **y** would represent nonspecific anti-cancer antigen antibody binding to normal cell components (**Ab1**), relative to signal intensity **x** representing primarily specific antibody binding to a housekeeping gene product (**Ab2**). Therefore, in a normal cell, the Nozawa ratio might be designated:

Nonspecific **Ab1** binding

Specific **Ab2** binding + nonspecific **Ab2** binding.

According to the present invention, in a normal cell sample, both components of the ratio represent primarily specific antibody binding:

Specific **Ab1** binding + nonspecific **Ab1** binding

Specific **Ab2** binding + nonspecific **Ab2** binding.

Here, the ratio is used to determine the normal levels of wild-type protein expression of the subject gene in normal cells.

To summarize, the use of a ratio of antigens in Nozawa et al. is different from that of the present invention, and is nonanalogous art. Applicant respectfully submits that there is nothing in Nozawa that adds to the disclosure of either Pece or Glendening that would render the present invention obvious.

Pece in view of Nozawa (Section 10 of Office Action

Claims 24-28, 32-35, 43, 44, 55-57, 61, 73, 74, 75, 79 and 80 stand rejected under 35 U.S.C. 103(a) as being "unpatentable over Pece (Pece, N. et al. J. Clin. Invest. 100(10):2568-2579, 1997, November; cited in IDS) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, page 4, Section 10]. Applicant respectfully points out that the instant rejection is moot as to claims 73, 74, 75, 79 and 80, which claims have been cancelled.

The Office Action states at page 5 that "it would have been prima facia obvious to one of ordinary skill in the art . . . to have made the claimed methods because Pece teaches that endoglin levels are reduced to 50% of normal in those with a predisposition for HHT1 or those actually affected by HHT1. . . " relying upon Nozawa for teaching "the motivation" for use of "methods for specifically quantitating antigens of interest by relating the amount of the detected antigen to the amount of a second antigen known in the art. . . . by describing many of the problems that occur when attempting to associate the detection of an antigen with a disease state. . . ." Applicant respectfully traverses that rejection first pointing out as detailed above under the sub-heading **Nozawa,** that Nozawa is not analogous art, and the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention. Nozawa certainly suggests nothing about the ratio of the amounts of wild-type proteins expressed by two or more subject genes, each known to be associated with a disease or a disease susceptibility trait in a single sample of normal cells from an individual. Applicant respectfully, but strenuously argues that Nozawa cannot provide motivation to Pece to use a ratio of wild-type proteins expressed from two or more subject genes in a single sample of normal cells from an individual according to the methods of this invention, in view of the numerous differences detailed above concerning the ratio, and the problems addressed by Nozawa in contrast to those of the instantly claimed invention.

Applicant respectfully further distinguishes the instantly claimed immunoassay methods from the methods of Pece, in that Pece does not suggest the use of a ratio of subject gene products, nor does Pece fully address "normal variation" of endoglin levels. The Office Action at page 5 admits that "Pece fails to explicitly teach making a ratio of protein levels of one subject gene to that of another subject gene."

First, Pece relates the endoglin levels in affected (hereditary hemorrhagic telangiectasia, or HHT) newborns directly to the endoglin levels in normal newborns, and finds a 50% reduction in endoglin in the affected newborns: "There were no detectable truncated forms of endoglin, and normal endoglin was expressed at half levels relative to control in all cases (Fig. 7 and Table I)." [Pece, page 2574, 2nd column.] In Figure 5B, Western blot analysis compares the densitometric units of endoglin levels found in the HHT newborn relative to a normal newborn, at equivalent human umbilical vein endothelial cells (HUVEC) lysate protein concentration. In Figure 7 and Table 1, quantitation of endoglin is determined by phosphorimager, calculating the patient/control ratio of pixel values of endoglin expression found by immunoprecipitation of patient samples. As Pece states in one passage cited by the Examiner: "Quantitative flow cytometry revealed that H19 HUVEC expressed 46±6% surface endoglin relative to the control HUVEC with all of the four mAb tested . . . suggesting that only normal endoglin is present at the cell surface." [Pece et al., 1997; page 2573, 1st column] No protein other than endoglin is probed or measured in the Western blots of Figure 5 and the immunoprecipitation experiments of Table 1 and Figure 7. The methods used by Pece imply that the primary consideration for gene dosage effects is the correct absolute level of gene product, in this case, correct absolute level of endoglin protein. As pointed out above in the response to the 112, first paragraph rejection, the instant invention is based on a different premise.

Whereas Pece measures $\alpha 5\beta 1$ integrin and CD31 levels in the flow cytometry analysis of Figure 4, Pece only relates the levels of proteins found in the affected individual to levels of the same proteins found in normal individuals: endoglin to endoglin, integrin to integrin, CD31 to CD31 (see, for example, Figure 4A, where control and HHT flow cytometry data are superimposed), suggesting that the latter two proteins are used to correct for sample loading. There is no use of integrin or CD31 levels in a ratio with endoglin levels. Moreover, there is no suggestion in Pece that there could or should be further refinement of the quantitative assays of endoglin, using a ratio of antigens (e.g., endoglin to CD31 ratio) found in one sample isolated from one individual, relative to the same ratio of antigens in normal individuals.

Secondly, there is an acceptable normal (non-deleterious) variation in products of genes subject to haploinsufficiency which Pece does not fully address. The sample from a patient newborn is matched with a sample from only one normal newborn; see, for example, Figure 5B. Pece does not indicate how to determine what range of endoglin expression may be acceptable in a newborn.

In fact, Pece screens two adult HHT patients for reduced endoglin expression in activated monocytes; perhaps because of a lack of adequate definition of "normal" variation in endoglin levels, Pece possibly overlooks endoglin as the source of HHT in two adult patients:

> H118 and H22 represent two clinically affected HHT patients, which were analyzed in parallel with H119, and two normal controls. . . . H118 and H22 were found to have normal levels of endoglin (91±16% and 84±19%) relative to the mean of the two controls. These values represent variations within normal values of endoglin and are clearly distinct from that of the HHT1 patient, H119 (42±9%). H118 and H22 are likely HHT2 patients but this will be confirmed by mutation analysis.

[Pece et al., page 2574, second column, second paragraph; emphasis added.] Pece does compare the affected patients H118 and H22 to two normal controls, and concludes that patients H118 and H22 have "normal" levels of endoglin; but Pece does not indicate how to arrive at an estimation of "variations within normal values of endoglin" beyond the two controls; also, if one looks at the values of endoglin found in patients H118 and H22, the lower values (within standard deviation) are 75% and 65% of "normal" average levels, respectively.

In contrast, the instant invention specifically addresses individual but normal (non-deleterious) variation in products of genes subject to haploinsufficiency:

> After applying these methods to (a) a normal population and (b) to a population of verified HNPCC cases, the immunoassay methods of this invention allow for the setting of criteria that enable one to distinguish between normal and abnormal levels of expression of full-length MMR proteins. The methods rely on the ratio of the expression level of the subject wild-type gene product of interest and that of a reference gene product, and the calculation of a ratio of one to the other.

[Instant application, page 6, lines 10-15.]

> As indicated above, the expected reduction in the cellular levels of full-length protein in the case of a truncation-causing mutation or a mutation causing allelic loss would be a 50% reduction in the amount of full-length protein expressed by a subject gene. . . . However, due to biological variability, a reduction in full-length protein expression from normal (for a single allelic mutation) could be considered herein to be a positive result, according to a threshold level that is statistically determined from the range of normal values in disease-free individuals from the general population. . . . Variability from those theoretical baselines for positive results could be ± 20%, preferably ± 15%, more preferably ± 10%. . . . When the assay results indicate that the subject gene has a mutation in one allele, the abnormally low amount of wild-type protein is generally about 50% of the control amount, with the above-noted variability being operative.

[Instant application, page 9, line 22 to page 10, line 2 and page 11, lines 19-21; emphasis added.]

Pece describes the product of the endoglin gene, subject to haploinsufficiency, as a component of the TGF-β1 receptor complex and also mentions a second HHT-associated gene, ALK-1, another member of the TGF-beta receptor family of proteins and possibly "involved in a common pathway controlling vasculogenesis and/or angiogenesis." [Pece, page 2569, column 1, second paragraph.] It was known at the time of Pece that ALK-1 is also subject to dominant autosomal germline null alleles associated with HHT [Berg et al., Am. J. Genet., 61(1): 60-67 (1997); citation on accompanying Form PTO A820, and abstract enclosed], but Pece does not suggest determining a ratio of endoglin to ALK-1 in families with HHT. It is possible, as ALK1 and endoglin are reported to associate together in a receptor complex on the surface of endothelial cells [Marchuk et al., Curr. Opin. Hematol., 5(5): 332-338 (1998); 3/9/04 IDS], that the source of the HHT disease in patients H118 and H22 is an abnormal endoglin-to-ALK1 protein ratio, which was not suggested nor discussed by Pece.

As indicated and detailed above, the Applicant relies upon the remarks in the previous Amendment and Response to Office Action dated March 3, 2004, concerning why Nozawa is nonanalogous art, how the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention, and how the problems addressed in Nozawa are very different from those addressed by the instant invention. Applicant respectfully concludes that neither Pece alone nor in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully requests that the Examiner reconsider the instant rejection in view of the above remarks and withdraw the instant 103(a) rejection.

Glendening in view of Nozawa (Section 11 of Office Action)

Claims 24-28, 32-35, 37-39, 43, 44, 55-57, 61, 73-77, 79 and 80 stand rejected under 35 U.S.C. 103(a) as being "unpatentable over Glendening (Glendening, J.M. et al., Cancer Res., 55: 5531-5535, 1995) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, pages 5 to 6, section 11]. The Applicant respectfully points out that the instant rejection is moot as to claims 73-77, 79 and 80, which claims have been cancelled.

The Office Action admits at page 6 that

> Glendening fails to teach methods where normal cells from patients are used for measurements of p16INK4 protein levels and also fails to explicitly teach making a ratio of protein levels of one subject gene to that of another subject gene.

However, the Office Action at page 6 finds that "it would have been prima facie obvious . . . to have made the claimed methods, because Glendening teaches that haploinsufficiency of p16INK4 appears to be a factor in the development of melanoma . . . " relying again upon Nozawa for "the motivation" to use "methods for specifically quantitating antigens of interest by relating the amount of detected antigen to the amount of a second antigen . . ." in that Nozawa describes "many of the problems that may occur when attempting to associate the detection of an antigen with a disease state. . . ." Again, Applicant respectfully traverses that rejection in that Nozawa, as

detailed above under the sub-heading **Nozawa**, is not analogous art, and the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention. Nozawa certainly suggests nothing about the ratio of the amounts of wild-type protein expressed by two or more subject genes, wherein each is known to be associated with a disease or a disease susceptibility trait, in a single sample of normal cells from an individual. Further, the problems addressed in Nozawa are substantially different from those addressed by the instant invention.

The Office Action at page 6 states in part:

> Glendening teaches that deletion of a single copy of p16INK4 appears to be enough to initiate or drive the development of melanoma (page 5535, 1st col). . . . Glendening teaches that in cells showing p16INK4 mutations, p16INK4 is still expressed indicating that there is haploinsufficiency (see abstract and page 5532, 2nd col).

Applicant respectfully distinguishes the instantly claimed immunoassay methods from the methods of Glendening, which are inapposite to the subject invention.

First, Applicant respectfully points out that Glendening is directed to the $p16^{INK4}$ gene in one sporadic melanoma patient (DX), and not to a patient with a germline mutation. While the Examiner points out that Glendening teaches that "germline mutations within the p16INK4 gene have been described for families where melanoma is common," there are many instances of diseases where the etiology of a sporadic disease is different from that of its hereditary counterpart. For example, mutations of the proto-oncogene RET have been shown to be involved in the pathogenesis of hereditary medullary thyroid carcinoma (MTC), whereas the etiology of sporadic MTC is apparently different, with indications that RET mutations appear in sporadic tumors only during subsequent clonal evolution. Another example can be found in chromophobe renal cell carcinoma (RCC); mutations in the folliculin gene (BHD) are associated with hereditary, but not sporadic, forms of this disease. [Nagy et al., Int. J. Cancer, 109(3): 472-475 (2004); citation on accompanying Form PTO A820 and abstract enclosed.]

Second, the only protein data provided in Glendening is from six cancer cell lines derived from the single sporadic melanoma patient (relative to levels in normal

melanocytes or unrelated melanomas), not protein data from normal cells from patients with hereditary melanoma.

Third, the protein data in Glendening is descriptive, not quantitative, and include the possibility that the protein levels of the sporadic melanoma patient lie within normal ranges for unaffected individuals, and not necessarily a 50% reduction, as indicated by the Examiner. As described by Glendening, "[i]n comparison to the expression of $p16^{INK4}$ protein in other unrelated melanoma cell lines and cultured melanocytes . . . , the levels in these cell lines were judged to be low to moderate" [Glendening, page 5532, col. 2]; but "these cell lines" of Glendening were all derived from one patient, DX. Clearly, at least one melanoma cell line independently derived from patient DX expressed "moderate" amounts of $p16^{INK4}$ protein, and "moderate" encompasses "average". Glendening indicates that normal cells derived from the same patient (lymphoblast line DX-B) have the full complement of the p16 gene, but does not show protein data from the DX-B line as a control in the Western analysis of Figure 4.

Fourth, Glendening does not teach how to overcome the confounding variables associated with immunoassay testing of haploinsufficiency, as does the instant invention, by using a ratio of wild-type proteins expressed by each of two genes in one individual.

Even if the $p16^{INK4}$ protein levels were quantitatively measured to reflect a consistent 50% reduction of p16INK4 protein in melanoma cells of a sporadic patient, as measured by a 50% reduction in the ratio of $p16^{INK4}$ protein to a second protein, the conclusions drawn from this protein data would not be applicable to normal cells in patients with hereditary melanoma.

Furthermore, the $p16^{INK4}$ situation is inherently more complicated than most disease-causing mutations. As explained in the instant application, the "immunoassay methods of this invention are...premised on the assumption that germline mutations in two different genes of one individual are very rare." [Application, page 5, line 24 to page 6, line 2.] In contrast to most genes associated with diseases, in which each discrete gene encodes only one protein, the $p16^{INK4}$ locus shares exons with a second gene, ARF; the locus alternately encodes two distinct products which function in two distinct pathways: the $p16^{INK4}$ protein, a cyclin-dependent kinase

inhibitor that functions upstream of RB (retinoblastoma), and the p19ARF protein, which blocks MDM2 inhibition of p53 activity. [Chin et al., Trends Biochem Sci., 23(8): 291-296 (1998); citation on accompanying Form PTO A820, and abstract enclosed.] Moreover, the third gene implicated in melanoma, p15 (or INK4B), maps immediately next to the p16/ARF locus. Deletions of this chromosome 9p region are likely to include all three genes, rather than only one.

Therefore, even if haploinsufficiency of the p16INK/ARF locus were demonstrated in hereditary cases of melanoma, it would be extremely difficult to identify the cause as reduction of the $p16^{INK}$ product, reduction of the ARF product, or reduction of both. If a ratio of $P16^{INK4}$ to ARF were used according to one embodiment of the present invention, even if the deletion were present, the ratio would most likely indicate no reduction of proteins, as both proteins would be reduced equally.

Although Glendening states that "deletion of a single copy of the $p16^{INK4}$ gene would appear to be enough to potentially initiate or drive the development of melanoma" (Glendening, page 5535, column 1), Glendening is referring to one sporadic melanoma patient. Additionally, according to Glendening, redundancy in the genome (expression of $p15^{INK4B}$, other cyclin dependent kinases, etc.) apparently can compensate for germline mutations of $p16^{INK4}$ in hereditary melanoma. [Id.] Gruis et al. 1995 report that patients that are homozygous for a deletion of the p16 gene can either be viable or show no obvious signs of melanoma [Gruis et al., Nat. Genet., 10(3): 351-353 (1995); citation on accompanying Form PTO A820 , and abstract enclosed], showing that the relationship between mutations in p16 and hereditary melanoma is not understood.

In summary, the instantly claimed methods can be distinguished from the methods of Glendening as follows:

(1) a single normal biological sample is assayed in the instantly claimed immunoassay methods, whereas the Glendening samples are derived from melanomas;

(2) the instantly claimed invention concerns detecting a germline mutation in a subject gene or in one of two or more subject genes, wherein the germline mutation is associated with a disease or a disease susceptibility trait, whereas Glendening describes data only for sporadic melanoma;

(3) Glendening does not suggest the use of a ratio of levels of two proteins within a single sample to correct for confounding immunoassay variables that are addressed by the instantly claimed methods; and

(4) the instantly claimed invention is based on the premise that germline mutations in two different genes of one individual are very rare, and as described by Glendening, germline mutations of $p16^{INK4}$ associated with hereditary melanoma appear to also be closely linked with germline mutations of ARF and p15INK4B .

Whereas Nozawa teaches the use of a ratio of antigens in their system, the use of a ratio of antigens in Nozawa is substantially different from that of the present invention. Nozawa is nonanalogous art. Applicant again respectfully relies upon the remarks in the previous Amendment and Response to Office Action dated March 3, 2004, concerning how the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention, and how the problems addressed in Nozawa are very different from those addressed by the instant inention. Moreover, there is no motivation provided in Glendening et al. to use a ratio of antigens .

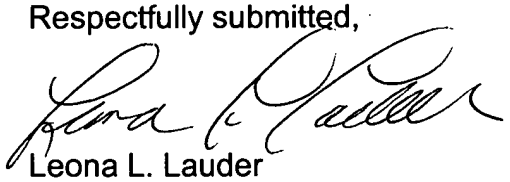
Applicant respectfully concludes that neither Glendening alone nor in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully requests that the Examiner reconsider the instant rejection in view of the above remarks and withdraw the instant 103(a) rejection.

## CONCLUSION

Applicant respectfully concludes that the claims as amended are in condition for allowance, and earnestly requests that the claims be promptly allowed. If for any reason the Examiner feels that a telephone conference could be helpful, the

Examiner is invited to telephone the undersigned Attorney for Applicant at (415) 981-2034.

Respectfully submitted,



Leona L. Lauder
Attorney for Applicant
Registration No. 30,863

Dated: November 15, 2004